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USE OF UK114 IN THE TREATMENT OF LEISHMANIASIS

This invention relates to the use of the protein UK114, possibly associated with ubiquitin, to treat leishmaniasis in humans and animals.

The protozoa of the *Leishmania* genus are intracellular parasites of the macrophages and dendritic cells of the dog, man and numerous wild animals. On the basis of the classification criteria used in human medicine, leishmaniasis presents in three clinical forms: visceral (known in man as "kala-azar"), cutaneous and mucocutaneous.

In the case of the dog, a cutaneous and a visceral form were separately classified in the past because of the characteristic clinical picture, but they are now both regarded as progressive forms of the same disease, known as "generalised canine leishmaniasis".

The vector is a sand-fly of the *Phlebotomus* genus in the Old World and the *Lutzomyia* genus in the New World. The protozoa multiply in the sand-fly and are transformed into infectious organisms.

Parasites of the *Leishmania* genus appear as rounded or oval organisms in the macrophage, with the rod-like kinetoplast adjacent to the nucleus. The organism, which measures 2 to 5 μm in diameter, possesses a rudimentary flagellum that does not extend beyond the edge of the cell. This **amastigote** form of the parasite is ingested by the sand-fly during the blood meal. The protozoon is transformed in the intestine of the intermediate host into the **promastigote** form, characterised by a long free flagellum that protrudes from the anterior extremity of the parasite. The organism has an elongated shape and can grow to a length of 15 μm, excluding the flagellum, which usually has the same dimensions as the body.

The amastigotes ingested reach the intestine of the sand-fly, where they are transformed into promastigotes. The promastigotes divide repeatedly by

binary fission, and subsequently migrate in the anterior direction. In the pharynx, the parasites turn into highly mobile metacyclic promastigotes, which migrate towards the proboscis. The promastigotes are transmitted to the new vertebrate host by means of the sand-fly's bite.

In the vertebrate host, the promastigotes are ingested by the monocytes/macrophages. After being ingested, the promastigote turns into an amastigote. The amastigotes divide by binary fission in the parasitophorous vacuole until their number is sufficient to rupture the macrophage. The amastigotes thus released are ingested by other macrophages.

The ability of the amastigotes to survive in the macrophages and spread throughout the body depends on factors intrinsic to the parasite and on factors associated with the type of cell-mediated immune response developed by the host. If parasitic macrophages are sufficiently stimulated by T-helper (Th) lymphocytes, they produce numerous lysosome enzymes and other factors including oxygen metabolites, hydrogen superoxide and peroxide and nitrous oxide (NO), which are toxic to the parasite.

The type of cell-mediated immune response and interleukin (IL) profile produced determine resistance or sensitivity to *Leishmania* infection. In laboratory animals, resistance to *Leishmania* infection is characterised by the **Th1** response, with production of IL-12 and interferon gamma (IFNγ) and activation of the macrophages which eliminate the parasite. Conversely, in animals sensitive to infection, the response is type **Th2**, characterised by production of IL-4 and IL-10 with consequent suppression of the parasiticidal activity of the macrophages and stimulation of the B lymphocytes with an increase in the production of immunoglobulins.

The humoral immune response in leishmaniasis is impressive, but not protective. The specific antibodies produced against *Leishmania* have no neutralising action against the parasite.

In animals sensitive to the disease the protozoon spreads throughout the body, in the macrophages. The parasite has been observed in all the organs and tissues of the body except the central nervous system. Slow, continuous contact between the parasitic antigen and the immunocompetent cells forms the basis for the pathogenetic development of the disease, which is characterised by:

hyperglobulinaemia, generally polyclonal, associated with continual stimulation of the B lymphocytes, which causes an increase in total proteins and inversion of the albumin/globulin ratio;

production of auto-antibodies, probably due to a cross-reaction between parasitic antigens and self-antigens, causing thrombocytopenia and anaemia;

production and deposit of immunocomplexes responsible for the vasculitis, glomerulonephritis and polyarthritis syndromes.

The pathogenesis of the skin lesions present in most sufferers is not yet clear. According to some authors, the persistence of the parasite in the macrophage continually stimulates infiltration by inflammatory cells, especially plasma cells, macrophages and lymphocytes, into the dermis. According to other authors, the deposit of immunocomplexes is the main cause of dermatitis which, on histological examination, often presents lesions similar to those caused by other diseases induced by immunocomplexes, such as systemic Lupus erythematosus. Finally, the skin alterations may be the result of vasculitis.

The symptoms of canine leishmaniasis are highly variable, and may include peripheral lymphadenopathy (over 90% of infected subjects), skin lesions (>80%), chronic conjunctivitis (50%), onychogryphosis (40%), anorexia (>35%), increased appetite (30%), weight loss (30%), fever (20%), kidney failure (20%), epistaxis (10%), uveitis (8%) and gait disorders (6%).

The skin signs are among the most important in the disease. Various

types of macroscopic and microscopic lesions have been described in canine leishmaniasis: dry exfoliative dermatitis, ulcerative dermatitis, nodular dermatitis, sterile pustular dermatitis, paronychia, and nasal and/or digital hyperkeratosis. The skin lesions are generally chronic, symmetrical and not itchy.

Recent studies clearly demonstrate the existence of a TNF-independent compensation mechanism able to activate the macrophages in the anti-leishmania response. As the **Th1** response mediated by interleukin-12 (IL-12) and interferon gamma (IFNγ) is paradoxically responsible not only for activation of the macrophages, but also for nearly all the symptoms of leishmaniasis, it may be advantageous to boost this compensation mechanism by inhibiting the **Th1** response.

The current elective treatment, based on antimony gluconate administered by infiltration (in cutaneous l.) or injection (in the other forms) can cause toxic effects (nausea and vomiting) sufficiently serious to require discontinuance of the treatment, which is replaced by treatment with aromatic diamines such as pentamidine, whose tolerability is generally poor.

It has now been found that the protein with molecular weight 14 kDa in SDS-PAGE, obtainable by extraction from mammal liver with perchloric acid, called **UK114** and disclosed in EP 574394 and US 5792744, is useful in the treatment of leishmaniasis, possibly associated with ubiquitin (**UK110**).

Recombinant protein UK114 is known from WO 00/63368.

Subcutaneous administration of **UK114** and ubiquitin to a group of 10 dogs with manifest clinical symptoms of leishmaniasis (peripheral lymphadenopathy and skin lesions of a high degree, mainly represented by sores and bleeding ulcers with loss of substance, anorexia and weight loss), at the doses and times indicated in the table, led to complete healing of all the lesions during the treatment period.

No adverse effects were observed during the treatment.

These findings demonstrate that the administration of **UK114** and ubiquitin cures the clinical symptoms of leishmaniasis in a totally safe manner. This is very interesting in view of the high toxicity of the drugs currently used in treatment, and the possibility of a response that is sometimes unsatisfactory from the clinical standpoint.

TABLE

Patient's weight < 10 kg	**1mg/day** subcutaneously for **6** days 7th day: rest **1 mg/day** subcutaneously for **6** more days
Patient's weight > 10 kg	As above, but doubling the dose: **2 mg/day**

According to the invention, protein UK114 of extractive or recombinant origin will therefore be opportunely administered to subjects suffering from leishmaniasis by the parenteral route, in particular subcutaneously or intramuscularly, at doses ranging between approx. 0.5 and 10 mg a day, until the disappearance or substantial reduction of the symptoms. The compositions according to the invention, in the form of solutions or suspensions in preferably aqueous sterile solvents, may also contain ubiquitin in a quantity corresponding to 0.1-5 mg per unit dose.

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